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MONITORING OF NITRATES IN RIVERS, STREAMS AND WASTEWATER OCCURRENCE AND EVALUATION OF SESSIONAL VARIATION

BY

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DEDICATION

I would like to dedicate my work to a number of people both in existence and none existence; my mother who is the epitome of unconditional love, selflessness and all round positiveness. My brother whom I love dearly and live to set examples for. My late father, a stand up, principled man to whom I aspire to be like. Lastly my kids, should I one day have the privilege of being a parent; want them to know dad always Went the extra mile even when he did not have to.

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ABSTRACT

The contamination of water bodies by chemicals such as nitrates have negative impact on water quality living organisms (aquatic organisms, animals and humans). Some communities in the developing countries solely rely on untreated river water as their only source of potable water. This is the case for the large number of South African citizens. Nitrate contamination is a serious problem in various water systems across the world. Therefore, this study investigated nitrate concentrations and physico-chemical parameters in surface water and wastewater samples from Umlaas River, Sterkspruit Stream and Hammarsdale Wastewater Treatment Works, South Africa. The monitoring of nitrates, pH, chemical oxygen demand, electrical conductivity and suspended solids was carried out seasonally (12-month cycle). The Chemical analyses indicated the nitrate concentrations was 0.10-6.10 mg L^{-1} , 0.30-21.4 mg L^{-1} and 0.31-28.1 mg L^{-1} for Umlaas River, Sterkspruit Stream and Hammarsdale Wastewater Treatment Works. The variation of on the concentration of the nitrates in waters samples across the four seasons was noticed. These concentrations were below the WHO guideline limit of 50 mg L^{-1} nitrates in all seasons. However, highest concentration of nitrates (21-28 mg L^{-1}) exceeding South African, European Union and USEPA guidelines were detected Sterkspruit Stream and Hammarsdale Wastewater Treatment Works during summer and autumn. Addition, Sterkspruit Stream was found to impacted by the effluent from Hammarsdale Wastewater Treatment Works. Electrical conductivity (EC) followed the order Sterkspruit Stream (52-195 mS/m) <Hammarsdale Wastewater Treatment Works (151-286 mS/m) < Umlaas River (24-333 mS/m). Notably, highest EC (144-333 mS/m) and chemical oxygen demand (470-536 mg L^{-1}) in water collected form Umlaas river during autumn (April and May).

The nitrates were evaluated for the potential health risk assessment to men, women and children surrounding the study area were from intake of nitrate containing water. The average daily dose (ADD) and hazard quotient (HQ) values were calculated based on nitrate concentrations in river water. The HQ values were < 1 for all seasons suggesting that there is no possibility of hazardous health problems in the study area. Additionally, parameters such nitrates, pH, chemical oxygen demand, electrical conductivity and suspended solids were use used to assess water quality using the water quality index (WQI) formula. The calculated WQI values are between 62.5-650 and 140-197 for Umlaas River, Sterkspruit Stream, respectively. The WQI values revealed that Umlaas River had good

quality water during summer and winter seasons while during autumn and winter seasons the water was poor and suitable for drinking. While in Sterkspruit Stream the water quality was poor in all seasons. Furthermore, the most effective water quality parameters on the determination of WQI for the present study were COD and EC.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

µg/g	Microgram per gram -
Ag^{+}	Silver cation
Br^{-}	Bromine-
DA	Discrete analyzer
EPA	Environmental Protection Agency
Fe^{2+}	ferrous cation
Fe^{3+}	Ferric cation
FIA	Flow injection analysis -
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-performance liquid chromatography
I^{-}	Iodide ion
ISO/IEC	International Organization for Standardization
K^{+}	potassium ion
kg/ha/yr	Kilogram per hectare per year
mg L^{-1}	Milligram per litre-
NH_4^{+}	ammonium ion
NIEC	Negative ion electron capture
NO_3^{-}	Nitrates
NO_3-N^{-}	Nitrate nitrogen
SANAS	South African National Accreditation System
SO_3^{2-}	Sulfate
UPLC–ESI/MS	Ultra-performance liquid chromatography–mass spectrometry
UV-vis	Ultraviolet–visible spectrophotometer
WHO	World Health Organization
WWTP	Wastewater treatment plant

CHAPTER 1:
INTRODUCTION

1.1 BACKGROUND AND JUSTIFICATION OF THE STUDY

Groundwater supplies approximately 65% of South Africa's rural drinking water [1-4]. In extension, majority of towns in South Africa depend on groundwater as a source of portable water [5-7]. Some parts of the world have the nitrate pollution predicament to such extremes that their water supplies have been officially declared unfit for drinking [8]. This was a real life case that happened in rural California in America [9]. In such cases, people depend solely on bottled water for meal preparation and drinking. This was a common practice throughout the community of rural California including schools and hospitals [10]. South Africa is a third world developing country, with a great deal of financial constraints. Bottled water could be very expensive when used as a permanent alternative for cooking and drinking because a lot of South African households still continue to live under the poverty line [11, 12]. Moreover, South Africans residing in urban areas uses treated water for domestic purposes. The source of this water is mostly from the water storage dams. However, there is still high population of South Africans that resides in rural communities which only rely on untreated river water as their source of portable water [13, 14].

Nitrogen-containing rocks are found in many parts of the world. The primary source of nitrogen in rocks is organic matter that is heavily loaded in sediments [15-17]. Alternatively, nitrogen can stem from thermal waters as a mixture of sedimentary, mantle and meteoric origin. Nitrogen can be incorporated into rocks as organic matter (for example, in carbonaceous shale), or as ammonium (NH_4^+) fixed in silicate minerals [17]. It is a common occurrence every year for substantial amounts of nitrogen to be inducted to most soils via precipitation. This nitrogen is regularly in a form of nitrate and ammonium, which are both customarily washed out of the atmosphere by precipitation. Much of the NO_3-N in the atmosphere originates from combustion, so the concentrations of nitrates issignificantly higher downwind from power plants or major industrial areas [18].

Humans play a detrimental role in nitrate pollution in surface and ground water sources. Nitrates contamination emanates from either point or non-point sources of pollution. The agricultural sector has contributed immensely to nitrogen contamination in surface waters due to the

utitilisation of nitrogen fertilizers to meet the consumer demands. Nitrogen compounds are used enormously in industrial settings. Some of the predominant nitrogen compounds used in industry are, anhydrous ammonia, nitric acid, ammonium nitrate and urea. A few of the industrial uses for nitrate include, manufacturing of plastic, metal processing, raw material in the textile industry, pulp, paper and rubber production and household cleaners. Nitrate contamination may result from careless and improper handling, disposal and use of these compounds, and the levels of contamination depends on the sources [10, 18]. The occurrence of nitrates in the aquatic resources poses health risks as these have been linked with various diseases such as methemoglobinemia disease found in infants [19-22]. Therefore, it is crucial to monitor the occurrence of nitrates regurlaly in water resoures such as rivers and wastewater treatment plants (WWTPs). Water in most South African rivers is used by widestock which are important food sources for the citezens. While, the treated wastewater from WWTPs is discharged into the rivers.

Nitrates are water soluble, hence, it is important to monitor their presence in WWTP effluents prior to devising stratergies for their complete removal from water resources. Water is an important resource for sustaining life since it is utilised for numerous activities in our everyday lives. The availability and access of water is generally a privilege but the availability of quality water should be the pinnacle of living species on earth [23]. There is a clear and obvious correlation between the quality of water and human and animal health. Studies suggest that 78% of fresh water comes from surface waters in a form of streams, rivers, lakes, reservoirs, and wetlands [24]. However, anthropogenic activities have led to the contamination of these surface waters. The presence of nitrates (NO_3^-) in surface water is observed as a challenge, this is because nitrates are listed as the second modest common pollutants of surface and ground waters [1, 3, 7, 9, 25, 26]. Nitrates and nitrites are two essential species involved in the nitrogen cycle, which is one of the most pivotal biogeochemical cycles in the aquatic space [27].

Based on this background, it is essential to monitor the occurrence of nitrates in water resources which include river water and WWTP effluents. This is more important in South Africa, where millions of residents solely depend on untreated surface water for their domestic needs which include consumption. In addition, livestock which is the main source of their meat and/or food consume the same untreated water.

1.2 PROBLEM STATEMENT

Nitrate contamination is a serious predicament in various water systems across the world [10, 26, 28]. Nitrates are nitrogen-oxygen chemical units which generally mix with various organic and inorganic compounds. Nitrates are nonvolatile and water soluble, thus making them difficult to escape water bodies [29]. Therefore, water systems become an intermediate medium for the existence of nitrates until induced by humans, animals and a whole host of different organisms [20]. Nitrates pose a health threat to humans and other living organisms [26]. Infants can contract the methemoglobinemia disease, particularly at elevated nitrate concentrations higher than 11 mg L^{-1} [20-22]. The disease is oxygen-depleting within the body since it consumes oxygen thus slowing down the transfer of oxygen to the tissues. Thus, nitrates can lead to oxygen deficiency and can cause a condition called cyanosis, where the lips and human excreta turn gray or blue [30]. In addition, nitrates can be converted to nitrite, which then interferes with the oxygen-carrying capacity of blood and the symptoms include shortness of breath and blueness of the skin [31] where symptoms can occur rapidly over a period of days. A prolonged exposure to higher levels of nitrate in water can be detrimental. Previous studies have suggested that the effects can even stretch as far as being carcinogenic; cancer types include gastric, colorectal, bladder, urothelial, brain, esophagus, ovarian, and non-Hodgkins lymphoma [32]. There is also some evidence to suggest that exposure to nitrates in drinking water is associated with adverse reproductive outcomes such as spontaneous abortions, intrauterine growth retardation, and various birth defects [20-22]. Death is a probable outcome in the event where the methemoglobinemia or blue baby disease is left untreated. Different parts of the world conform to different limitations for nitrate concentration in water. The concentration levels of nitrates in different water sources need to be closely monitored and restricted to safe limits to ensure human and animal safety. Seasonal monitoring of nitrates in rivers, streams and wastewater is pivotal in order to foresee possible future outbreaks and abnormalities which could be health threatening. With this said, the monitoring of nitrates in the water is therefore imperative and necessary as a health and safety precaution.

1.3 AIMS AND OBJECTIVES

The main aim of this study was to monitor the nitrate concentrations in water sources, i.e. rivers, streams, and wastewater samples collected from different sites in the KwaZulu-Natal region. The variation of nitrate concentrations in water sources was monitored seasonally, i.e. spring, summer, autumn, and winter).

Objectives:

- To quantify nitrate concentration in one specific river, stream, and WWTP.
- To investigate the variations of nitrate concentrations in KwaZulu-Natal water resources over different seasons.
- To investigate the environmental and health impacts associated with ungoverned levels of nitrates in water bodies

1.4 HYPOTHESIS

Nitrate concentrations vary wildly from season to season as a result of transmutation in climate and weather. Within a season, there should be a correlation between the weather patterns and the nitrate concentrations, relative to the rest of the seasons of the year. All these seasonal changes should have a significant impact on the characteristics of the water and nitrate concentrations.

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CHAPTER 2:
LITERATURE REVIEW OF METHODOLOGIES FOR DETECTION AND REMOVAL OF NITRATE FROM WATER MATRICES

2.1 SOURCES OF NITRATES

2.1.1 Geogenic Sources

2.1.1.1 Lightning Storms

During the lightning storms, the atmospheric nitrogen is turned into ammonia (Shukla et al 2018). Ammonia is then converted to nitrate and subsequently deposited into soil, groundwater, and surface water via rainfall and other precipitation modes (Shukla et al 2018)(Akwensioge et al 2012). Insight of the parameters of nitrates in surface water, it should be expected that results of relatively high levels of nitrates in water be observed during the stormy rainy seasons of the year (Fahrner et al 2002).

2.1.1.1 Geological origin

Nitrogen-containing rocks are found in many parts of the world. In the past, minimal attention has been directed on investigation their contribution to nutrient cycling. It is only in recent studies where special attention has been dedicated in these rocks and acknowledging their great potential pool of nitrogen [33]; [28]). The primary source of nitrogen in rocks is organic matter that is heavily loaded in sediments[17] (). Alternatively, nitrogen can stem from thermal waters as a mixture of sedimentary, mantle and meteoric origin. Nitrogen can be incorporated into rocks as organic matter (for example, in carbonaceous shale), or as ammonium (NH_4^+) fixed in silicate minerals[17]. The nitrogen that is accommodated in organic matter is transformed to ammonium during diagenesis, which in turn can be exchange for potassium (K^+) in silicate minerals. Ammonium end-member silicate minerals include buddingtonite, tobelite, ammonium muscovite and ammonium biotite. These processes may be more conspicuous in surface waters than in deeper groundwaters due to the phenomenon that weathering rate of rocks is higher under surface conditions, for example, natural geogenic nitrogen in stream water and thus the release of nitrogen[17]. Though, in some cases differentiating between human prompted environmental pollution and pollutants activities (anthropogenic) and environmental outcomes relating to the history of the earth

(geogenic) origin of nitrate in surface water can possess quite challenges and need to be observed closely and on a case-by-case basis.

Studies executed in early nineties in semi-arid regions of North America insinuate that it was not unusual for relatively large quantities of plant-available nitrogen to be present beneath root zones of native prairie vegetation[34]. In rare cases, concentrations of nitrate-N (NO_3-N) were as great as 36 µg/g soil 150 cm beneath native range in eastern Montana, at a time when very little of that land was cultivated[17]. It is very uncomfortable to think about after so many years of cultivation, how critical the situation is in present. These results hint that, in regions where relatively unweathered sedimentary deposits exist beneath the root zone, there is potential for the presence of residual exchangeable ammonium, which is readily oxidized to NO_3-N when exposed to proper conditions. An additional source of sub-soil NO_3-N accumulations may result from sub-surface seepage through perched water tables. Water and nitrates could leach through fallow sandy soils until they reach a permeable aquifer [17]. Nitrates would then flow essentially horizontally through the shallow aquifer and exit the soil by a hillside seep exemplified in the Republic of Malt. A concentration range of 50-100 mg L^{-1} NO_3-N of seep water is very common [18].

2.1.1.3 Precipitation

It is common every year for substantial amounts of nitrogen to be inducted to most soils via precipitation. This nitrogen is regularly in a form of nitrate and ammonium, which are both customarily washed out of the atmosphere by precipitation. Major agricultural sources of atmospheric ammonium are ammonia volatilization from soils, fertilizers, animal wastes and vegetation [18]. Studies show that considerable amounts of ammonia may decamp through stomata of plant leaves in the transpiration stream [35]. This process is particularly important during senescence of well-fertilized vegetation. Some of the ammonia escaping the soil and plant surfaces may be reabsorbed and utilized by other plant leaves, with the balance decamping to the atmosphere. The total aggregate of nitrogen added to the soil through precipitation is highly variable and is predicated on surrounding agricultural and industrial activities. In temperate regions and natural ecosystems where precipitation is the major source of nitrogen, the nitrogen quantity ranges between 10 - 14 kg/ha/yr [18].

2.1.2 Anthropogenic sources

2.1.2.1 Human and animal wastes

Humans and animals play a detrimental role in nitrate pollution in water sources in their respective spaces of land of occupancy [36]. Nitrates from such waste can display the characteristics of either point or non-point source pollution [37]. Point sources occur at or near the actual waste facility involved and typically display elevated degrees of nitrate or ammonia in a limited domain [38] (Zhao). Diffuse sources are spread over large areas (for example, in agricultural fertilization), and affected aquifers are often classified by lower (but ≥10 mg L^{-1}) levels of NO3-N. Nitrate from human waste emerge commonly from individual septic systems or municipal wastewater treatment facilities and pit latrines in rural areas [36]. Quintessentially, effluents from such septic systems are in the order of 30- 60 mg L^{-1} total nitrogen, with ammonia making up the majority of the nitrogen [39]. The nitrogen content of these effluents varies widely depending upon the condition of the individual system and the type of waste being introduced. A greater number of the population is serviced by municipal wastewater treatment systems. Nitrogen content of effluent from municipal systems will vary according to the nature of the incoming waste stream and the type and condition of the system. Depending on where the waste treatment plant is situated its specification defers, the plants inflow is influenced by the surrounding activities. Some treatment plants operate on a ratio of 90: 10 industrials to domestic waste while others on the basis of 85:10:5. A perfect epitome of this case is a WWTP in Mpumalanga, KwaZulu-Natal called Hammarsdale treatment works. It services a whole range of different industries which account for the 85% of their effluent, the 10% is contributed solely by a chicken company called rainbow chicken which deposits organic matter, and lastly 5% is contributed by domestic households. However, after primary treatment with activated sludge, the effluent typically still contains about 15-35 mg L^{-1} of total nitrogen. More advanced systems can reduce this to about 2-10 mg L^{-1} [7]. Waste from dairies, open feedlots, confined feeding operations, stockyards and other facilities for rising and holding animals is also a potential source of nitrate and other forms of nitrogen. The public is understandably vocal about their concerns and grievances over animal waste, namely odours issues and flies. In addition, the animal waste generally present serious nitrate pollution problems in surface and groundwater.

2.1.2.2 Fertilizers

Fertilizers are of crucial significance to support production of food through agriculture and aquaculture. Among all kinds of fertilizers, nitrogen containing fertilizers (N-fertilizers) are by far still the most utilized alternative method owing its popularity to its cost effectiveness. N-fertilizer usually takes one of two forms: inorganic fertilizer and animal waste. Inorganic fertilizer usage has become famous in the last half of the twentieth century with the advent of anhydrous ammonia, liquid nitrogen, urea and similar formulations that have greatly increased crop yields, for example, in Australia [40]; [41, 42]. The long-term fate of N-fertilizer in the soil-water-plant systems is not fully understood [43]. Studies have reported that even after three decades of N-fertilizers have been applied in the ground, about 12-15% in the soil organic matter, while more 10% N fertilizer is leaked to groundwater system, thus contamination drinking water sources [44]. Therefore, it could predicted that the portion that is residing in the soil fractions could be taken up by plants or leak to the water system.

2.1.3 Industrial uses of nitrate

Nitrogen compounds are used enormously in industrial settings [36]. Some of the predominant nitrogen compounds used in industry are, anhydrous ammonia, nitric acid, ammonium nitrate and urea [36]. Therefore, nitrogen in the aforementioned compounds is transformed to nitrite and nitrate. In addition, industrial activities such as manufacturing of plastic, metal processing, raw material in the textile industry, pulp, paper and rubber production and household cleaners uses nitrates. Nitrate contamination may result from careless and improper handling, disposal and use of these compounds, and levels of contamination will depend on the source [36])

2.2 NITRATE CONTAMINATION

There are numerous factors that contribute to nitrate contamination in water systems. An inference is drawn that agricultural practices contribute majorly to nitrate contamination in various water sources[45]. Agriculture is a big and lucrative business across the globe. The model of business is built around optimum production. The success of the business is predicated on how much could be produced and sold for profit. In attempts to reach projected annual revenues and profits, farms resort to using nitrogen-based fertilizers [46]. Generally, farming is a seasonal activity and globally the most common season for farming is spring. In many cases it is commonly observed that most nitrate pollution problems are encountered in

spring [46]. It is in this time frame that farmers apply nitrogen-based fertilizers on agricultural fields. Dissolved nitrates in run-off water containing nitrogenous animal waste (urine, faeces, uneaten food, decayed tissues, etc.) from poultry, dairy cattle, hogs and agricultural species, is also of great concern [47]. Manure storage is another associate predicament. It is a common agricultural practice for farmers to excavate large holes on the ground to store manure [48]. This is in general a relatively cheaper alternative for short periods of time. In hindsight, this is a direct gateway to excessive leaching of nitrates [46]. Processing water used to clean and prepare poultry and meat food products is also uncovered as a culprit in nitrate contamination [47]. This water is utterly laden with nitrates and is normally kept and utilized in crop irrigation. The crops in turn absorb nitrate and they are bound to wind up in humans and animals leading to diseases and illness. People residing in rural areas commonly use on-lot septic systems and some homeowners depend on lawn fertilizers. These too can be sources of nitrate in rivers, streams and treatment works water.

2.3 MAXIMUM ALLOWABLE LIMIT FOR NITRATES IN WATER

In the interest of human and animal health as well as safety and precautions need to be taken in order to ensure that nitrate concentration levels in water are limited to safe levels for consumption. The environmental protection agency, Ethekwini municipality and Sanas formulated standards have limits for nitrates in public water supplies [49]. According to these standards, the water supply should be less than or equal to 11 mg L^{-1} of nitrate-nitrogen [49]. Furthermore, as preventive measure against infantile methemoglobinemia the World Health Organisation (WHO), United States Environment Protection Agency (USEPA) and European Union (EU) have limits of nitrate in drinking water to be 50 mg L^{-1} , 44.3 mg L^{-1} and 11.3 mg L^{-1} respectivly [36]. In the African countries the WHO drinking water guidelines are implemented and each countrie's local water quality standards legislation are derived from WHO guideline. ISO/IEC 17025 standard stipulates the general requirements for competency of a testing and calibrating laboratory. In essence, it is an international reference for testing and calibrating laboratories seeking to demonstrate their capability to produce reliable results. In essence, the standard is in place to protect the health and safety of the public with regards to the supplied water for consumption and general use. It ensures that all the water consumed by the public is within specification and if not alter the necessary bodies to immediately attend to the problem to correct it, to avoid little to no exposure to the public.

2.4 OCCURRENCE OF NITRATES IN WATER SYSTEMS

According to previous studies nitrate concentrations below and above permissible limits in drinking water sources is the universal problem[50]. Various levels of nitrates have been detected in different water matrices across the globe (Table 2.1). According to [36], since 1990s the global levels of nitrate in waterways have increased by approximately 36%. The highest increase has been observed in Africa and Eastern Mediterranean[51]; [52]. The interesting part is that both developed and developing countries have recorded increasing occurrence of nitrate in water systems (Table 2.1). Even though developed countries are characterised by lower population density as compared to developing countries, still similar contamination trends in water resources have been reported [36].

[53], reported that 105 groundwater samples collected in rock-dominant semi-arid region in India contained nitrate concentration ranging between 12 and 212 mg L^{-1}. They observed that the northern and southern region of their study area had elevated nitrate concentrations as compared to the central region (nitrate concentration was below 45 mg L^{-1}) [53]. Furthermore, their findings revealed that 17% of the groundwater samples fall under a very high-risk categories while 55% fall under high risk[53]. These results suggested that 72% of the 105 ground water samples was suitable for drinking purposes. The authors alluded to the fact that the elevated levels of nitrates were due to N-fertilizer applications in the regions.

[54] published a review based on the presence of nitrate in Tanzanian groundwater aquifers. Their study revealed that nitrate concentrations reported in Tanzanian groundwater ranged from 100-478 mg L^{-1}. According to the authors these high concentration values could be attributed to various human activities such as onsite sanitation centres and agricultural activities [54]. A study in Romania [55] revealed that approximately 88% groundwater samples from Ozun village, contained high concentrations of nitrate (above 10 mg L^{-1}) while 75% did not comply with drinking water standards.[56] discovered that high concentration of nitrates up to 162 mg L^{-1} in shallow groundwater samples were as the results of fertilization and irrigation periods.

Based on Table 1, the highest concentration of 1500 mg L^{-1} [36] was found in Indian surface water. This concentration 1500 mg L^{-1} is 52 times higher/lower than the maximum level of 29 mg L^{-1} [57] (Dube) ever reported in South African surface water. Despite South African research having produced a lower nitrate level in surface water compared to India, the reported environmental concentration is still a concern since it exceeds the maximum allowable level of 10 mg L^{-1} recommended by South African regulatory bodies such as

SANAS. Due to the population growth in South Africa, communities need to increase their food sources. Therefore, the agricultural industry is likely to grow which could result in an increase in the usage of nitrate based fertilizer, thereby, causing high nitrate pollution loads in the environmental waters.

Table 2.1: Global concentrations of nitrates detected in various water matrices

Country	Detected concentration (mg L^{-1})			Reference
	Ground water	**Surface water**	**WWTP influent, effluent**	
China	0.59-824.80	-	0.6-12.1	[58],[59]
Greece	39.0	62.0		[60]
China	124.4		20-100	[61], [62]
USA		0.03-5.46		[63]
Iran	0.45-79.58			[64]
India	17-120		0.32-6.5	[65], [66]
Transboundary (Russia/Ukraine)	0.5-100	0.25-22		[67] 7
South korea	79.4		60-1000	[68] ,[69]
China	850			[70]
China Yantia	17.8			[71]
Germany	50			[72]
Spain		5.0	0.83±0.33	[73], [74]

Canada		45.0		[71]
Mexico	0.01-33			[75]
Mexico	2.36-18.13			[75]
South Africa		5.56-29	0.01-90	[57], [76]
Nigeria		1-770		[77]
Japan	82.4			[78]
Poland		1.2-6.0		[79]
Southern India		1500		[36]
Iran	8-33.7		20.0	[80], [81]
Iran		1-51		[80]
Iran		20-23		[82]
Europe	17.5			[20]
Russia	16-145			[83]
Iran		10.55		[84]
China	20.0			[61]
Iran	75.0			[85]
Canada	5-100			Canter, L.W., 2019
Oklahoma,United State	0.4-91			[86]
Nigeria		1000	12.89-211.2	[87], [88]
Mozambique	250			[89]
Ghana	0.12-733			[90]
Tanzania	1.1-357.7		2.35-9.19	[91], [92]
Tanzania	2.3-32.6			[91]
Japan	12.26		136.5-168.1	[93], [94]
Japan	4.5-8.0			[93]
Philippines	7.0			[95]
China	229.1			[61]

South Africa		1.428	0.59	Sphe 2020
South Africa		4.634	0.32	Sphe 2020
Mexico	80.0			de León-Gómez et al 2020
Egypt	418.0		0.01-7.88	[96], [97]
Jamaica	0.76-7.04			[98]
Ethiopia	17.0		1.3.0	[99], [100]
India	7.10-82.0			[101]
Dubai	1000		34.32-403	Alsharhan et al 2020, Al-[102]
Kenya	0.04-90.6		2.40 - 11.0	Nyilitya et al 2020, [103]
Kenya	20.0-70.0			Nyilitya et al 2020
Kenya	11.4-90.6			Nyilitya et al 2020
Argentina	4-235		9.83 ± 3.11	[104], [105]

2.5 ANALYTICAL METHODS FOR NITRATES IN WATER

2.5.1 Spectrophotometric methods

Spectrophotometric detection methodologies are the widely used techniques for determination of nitrates in water samples or any other samples [106]; [107]; [108]. UV-vis spectrophotometer is widely used due to its versatility, simplicity, and feasibility. This analytical technique is based on the measurement of absorbance corresponding to the nitrate concentration present in aqueous solution at the wavelength of 210 nm [109]. However, the presence of nitrite, iron (III), chlorine and organic matter interferes with nitrate detection because they tend to adsorb UV radiation at the same wavelength [109]. Efforts to avoid over estimation of nitrates using UV spectrophotometer have been reported in the literature[109]. In this case, the detection of nitrates or nitrites by spectrophotometric methodologies has been classified into three categories such as Griess assay [59, 110], nitrosation [111] and catalytic [112] methods.

Griess assay is one of the common methods for the detection of nitrates. This method involves the use of diazo-coupling procedure under acidic medium in the presence of nitrite and amine groups on a certain chromophore[113]. The chromophore is used to sense the colour changes taking place during the process and this subsequently lead to nitrite detection[113]. For example, (Valiente, N., Gil-Márquez, et al 2020) reported the application of Griess assay spectrophotometric method for analysis of nitrates in hypersaline natural samples. Lin et al. reported an automated Griess spectrophotometric method for simultaneous spectrophotometric determination of nitriles and nitrates in seawater samples [114]. Other recent methods are reported in the literature ([115] [59]; [116]; [117]; [118]).

Nitrosated based spectrophotometric method is based on reaction of nitrites with other indicating species, other than azo groups, to produce coloured solutions (Wang et al. 2017). For instance, barbituric acid in the presence of an acid to give violuric acid, a nitroso derivative (Wang et al. 2017). For this method, an analytical wavelength of 310-312 nm is used for the detection of nitrites [111]. Brienza and co-workers developed N-nitrosation spectrophotometric method for analysis of secondary amines in nitrate-rich wastewater[111]. Ding et al evaluated the possibility of using nitrosated based spectrophotometric method for analysis of nitrate and nitrite contents in pickled fruit and vegetable products [119]. Catalytic-spectrophotometric method is the most widely used procedure that is reported in the literature [120]; [121, 122]. This method is rooted on the catalytic effect of nitrite on oxidation of some

indicative species or organic dyes in the presence of a suitable oxidizing agents[123]. Several indicative species or organic dyes which include perphenazine, chlorpromazine, brilliant cresyl blue and methyl red have been reported in the literature [123]. Oxidizing agents that are widely used in catalytic-spectrophotometric methods are potassium permanganate, hydrogen peroxide, potassium bromate and potassium chlorate [123]. Like the previously described method (nitrosated based spectrophotometric method), a catalytic spectrophotometric method is also performed in an acidic medium. The disadvantages of catalytic spectrophotometric procedures is the fact that they are laborious and sufferer from interferences caused by the present of coaxing ions such as Fe^{2+}, Fe^{3+}, Ag^{+}, SO_3^{2-}, Br^{-} and I^{-} [123] . To overcome these challenges, researchers have reported the alternative methods such as the use of surfactants or using specific indicating agents, as well as the used flow injection analysis (FIA) [123]. Altunay et al. reported the application of catalytic spectrophotometric method based on the use of iodine, Triton X-114 and Coomassie brilliant blue R 250 for the sensitive indirect detection of trace nitrite, nitrate, and total nitrite in food samples (Altunay). Olgac reported the application of ion pair ultrasound assisted-cloud point extraction coupled with catalytic microvolume UV-Vis spectrophotometry for speciation of nitrates and nitrates beverage and food samples [124].

2.5.2 Colorimetric method: Discrete Analyzer

Discrete analyzer (DA) is an automated technique that combines photometric (that is colorimetric and enzymatic) and electrochemical (pH and conductivity) analysis [125]. It provides attractive features such as rapidity, robustness, flexibility, reproducibility and simplicity [125]. Furthermore, DA allows the simultaneous measurement of multiple analytes thus reducing total analysis and operation time [126, 127]; [128].

2.5.3 Chromatographic methods

Chromatographic techniques such as high-performance liquid chromatography (HPLC) and ion chromatography have been widely used for direct determination of nitrates and nitrites in different sample matrices [129]; [114]. Gas chromatography is seldomly used because it requires chemical modification, thus elongating the analysis time. This is because nitrates are not volatile. In recent years, GC-MS has been used for detection and quantification of nitrite and nitrate in biological samples [130, 131]; [132]; [133]. To achieve this, highly selective and sensitive ionization techniques such as in the negative ion electron capture (NIEC) is

used [130]; [131]; [132]; [133]). Campanella et al. reported the use of isotope dilution GC-MS for the determination of nitrates in vegetables (Campanella). Sample extracts were treated with with sulfamic acid to remove nitrites, while triethyloxonium tetrafluoroborate was used to convert nitrate into volatile species. The analytes were separated from sample in the form of gaseous derivative via headspace and the volatile derivative was detected using electron impact ionization. Other application of Gas chromatography–mass spectrometry (GC-MS) for analysis of nitrates in food samples has been reported in the literature [134]; [135]; [136].

The application of HPLC for rapid, selective and sensitive detection of nitrates have attracted a lot of attention ([114];[137, 138];[139, 140]; [141]; Khan 2016). This is because HPLC can be coupled with UV and electrochemical detectors. In a recent study, simultaneous determination of nitrate and nitrite in vegetables was achieved by using a monolithic capillary liquid chromatography coupled with UV detection [114]; [137]; [138];[139]; [140]; [141]). Moshoeshoe et al. reported the use of HPLC coupled with UV detection for simultaneous determination of nitrate, nitrite and phosphate in environmental samples [142]. Coviello et al. conducted a study on the analysis of nitrite and nitrate determination in meat foods for infants using Ion chromatography(IC) coupled with conductivity detection [143]. A fast, precise and sensitive chromatographic method for determination of nitrate and nitrite in drinking water was reported by [137]. In their study, ultra-performance liquid chromatography–mass spectrometry (UPLC–ESI/MS) was used for separation and quantification of nitrites and nitrates. Other applications of chromatographic techniques are reported in the literature[144]; [145]; [146]; [147]; [148]

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CHAPTER 3:
NITRATES, PHYSICO-CHEMICAL PARAMETERS AND HEALTH RISK ASSESSMENT OF RIVER WATER AND WASTEWATER TREATMENT PLANT OUTFALL IN KWAZULU-NATAL PROVINCE OF SOUTH AFRICA: SEASONAL TRENDS

ABSTRACT

Polluted surface water and wastewater can have serious impact on the human health and aquatic life. In this study, nitrates and physicochemical properties were monitored in surface water and wastewater samples collected from Umlaas River, Sterkspruit Stream and Hammarsdale wastewater treatment plant (WWTP) located in South Africa, over a period of one year. Potential human health risks due to nitrate contamination in river water were investigated. Nitrate concentrations ranged from <0.10-6.10, 0.30-21.4 and 0.31-28.1 mg L^{-1} in Umlaas River, Sterkspruit Stream and Hammarsdale WWTP, respectively. Based on these findings which resulted in hazard quotients of less than 1, there were no significant health effects posed by nitrates levels to men, women and children due to their access into the water in Umlaas River. Despite these results, water from Umlaas River was still deemed to be unsafe for human consumption due to the physicochemical parameters that exceeded the guidelines set by the World Health Organization (WHO). In this context, the highest COD value of 536 mg L^{-1} was found in Umlaas River which is much higher than the maximum allowable value of 4 mg L^{-1} set by WHO for drinking water. Therefore, treatment strategies are still required to reduce the organic matter and improve water quality. Furthermore the water quality index (WQI) and principal component analysis revealed that electrical conductivity and chemical oxygen demand which significantly affected the quality of water.

3.1 INTRODUCTION

The contamination of water bodies by chemicals have negative impact on all living organisms which include aquatic organisms and humans [149-156]. Some communities in the developing countries solely rely on untreated river water as their only source of potable water. This is the case for the large number of South African citizens [153, 157, 158]. In this case, humans share water in rivers for their basic needs with the livestock such as cattles, sheep and goats. Essentially, the contamination of such rivers should be prevented at all costs to safeguard the health of animals and humans. On the other end, wastewater treatment plants

(WWTPs) treat both domestic and industrial waste prior to discharging the treated water into the nearby rivers and in fewer instances into the open oceans [154, 159, 160]. This continues to happen despite the existing knowledge that WWTPs do not completely remove pollutants during the wastewater treatment process and allows such contaminants to enter into the surface water as part of effluents [161]. It becomes difficult to avoid the contamination of surface water in developing countries and/or rural communities due to the lack of modern ablution facilities which allows for the transfer of sewage into a designated area. In this case, sewage in the form of faeces and urine is directly deposited into the ground and swept into the rivers due to water run-off during the rainy days.

Nitrate contamination is a serious predicament in various water systems across the world [162, 163]. Nitrates are non-volatile and water soluble compounds, therefore, the wastewater treatment processes are unable to entirely remove them from water [164]. This becomes a crucial source of nitrates in the environment which poses health threat to humans and aquatic species. Other sources of nitrates in the environment are associated to the natural cycle and human activities, originating from uncontrolled land discharges of treated or raw domestic and industrial wastewaters, landfills, and animal wastes predominantly from animal farms [163]. Exposure to nitrates present in water, particularly drinking water is associated with adverse human health problems. These have been recently reviewed highlighting spontaneous abortions, intrauterine growth retardation, and various birth defects [20]. Besides these health problems, the presence of nitrates in rivers causes the depletion of aquifers and eutrophication [163]. Currently, South African tourism industry together with municipalities and government are trying to eradicate the enormous growth of water hyacinth plants which is believed to be induced by eutrophication. Water hyacinth plants grow and cover wide area over water in important tourist destinations such as dams. At the same time, these plants block-out sunlight from the aquatic life. In an attempt to reduce the health risks associated with the occurrence of nitrates in water, various organizations have set the maximum allowable limits of nitrates in important water resources. According to South African regulations, the amount of nitrates present in the public water supplies should not exceed 12 mg L^{-1} [153, 165]. World Health Organization (WHO) has set the guideline value of 50 mg L^{-1} for nitrates in drinking water [166].

Since nitrate pollution in water bodies causes health concerns, it is essential to monitor its occurrence in water regularly, especially in the water scarce regions. This is necessary as the quality of water is a public concern. Various researchers have indicated that the nitrate pollution levels in certain rivers exceed the healthy water standard of 50 mg L^{-1} set by WHO.

This has been reported in several rivers across different countries which include the Yellow River (China), Nakdong River basin (Republic of Korea) and River Thames (UK), with the nitrates concentrations found to reach 138.5 mg L^{-1} [167], 51.9 mg L^{-1} [168] and 69.6 mg L^{-1} [169], respectively. In the light of this information, health risk assessment of river impacted with nitrate contamination is essential.

To date, there is still a lack of information available in public domain on the occurrence of nitrates in South African water resources and WWTPs. However, the monitoring of nitrates and critical physicochemical parameters in South African aquatic environment remains essential as these are key indicators of water quality. This is of paramount important in South Africa, where millions of residents depend on untreated surface water for their domestic needs which include consumption. In addition, livestock which is the main source of meat and/or food in South Africa drink the same untreated water from rivers. Therefore, the aim of this study was to monitor the nitrate levels and physicochemical parameters in Hammersdale WWTP outfall and its effluent receiving water bodies. Based on the nitrate levels found in river water, human health risk assessment was performed to determine the implications of pollution on the community that depend on this water resource for their daily needs.

3.2 MATERIALS AND METHODS

3.2.1. Study sites and sample collection

In this study, nitrates levels and physico-chemical parameters were monitored in the outfall of Hammarsdale WWTP as well as in the effluent receiving water body (Sterk stream). Water flowing in the Sterk stream is channelled into Umlaas River which was also sampled. About 85% of wastewater that is being treated in Hammarsdale WWTP originates from different industries located in a small town of Hammarsdale in KwaZulu-Natal Province of South Africa. Some of these industries specializes in textile and chemical production, pulp and paper as well as leather processing. Hammarsdale WWTP also receives 10% of its wastewater from the chicken growing company (Rainbow chicken) and 5% from domestic sources. A total of 144 water samples were collected monthly over a year period from December 2017 to November 2018 to cover four seasons. An attempt was made to ensure that a representable sample was collected in each case. In the stream and river, three composite samples were collected from up, middle and downstream. These composite

samples were combined and transported into the laboratory. Some parameters were monitored upon arrival in the laboratory, while other tests were conducted within two days.

3.2.2 Water sample analysis

3.2.2.1 Physicochemical parameters

3.2.2.1.1 pH measurement procedure

Before use, the pH electrode was first calibrated with standard buffer solutions of known pH values that accommodates the range being measured. The pH measurements were carried by immersing the electrode into the sample solution until a steady reading was attained. The measurements were carried out in triplets to establish data precision and sensitivity. The electrode was then rinsed after each sample and stored in a storage solution after all the measurements have been completed.

3.2.2.1.2 Conductivity meter

The conductivity meter was set up for use following the manufacturer's guidelines. The conductivity standard solutions (typically potassium chloride or sodium chloride) were used to calibrate the meter for the range that will be measured. The probe is rinsed with distilled or deionized water. The appropriate range should be selected beginning with the highest range and working down. The conductivity of the water sample was measured by immersing the probe into the samples. If the reading is in the lower 10 percent of the range, switching to the next lower range is advised. If the conductivity of the sample exceeds the range of the instrument, the sample may be diluted.

3.2.2.1.3 Chemical oxygen demand (COD) Test Procedures

A series of known standards are prepared using KHP (potassium hydrogen phthalate). Most wastewater samples will fall in the high range, so standards of 100, 250, 500 and 1000 mg L are typically prepared. COD standards can also be purchased. A COD reactor/heating (150°C) block and a colorimeter are turned on so that both instruments are allowed to stabilize.Pre-prepared low-range (3-50 ppm) or high-range (20-1500 ppm) vials are selected for the COD test based on expected results. Both ranges can be used if expected results are unknown. One vial is marked as a “blank,” and three or four vials are marked with known standard levels. Two vials are then marked for the wastewater sample to make a duplicate

run. Note: If multiple wastewater samples are being run, at least 10% of samples are duplicated. 2 mL of liquid are added to each vial. In the case of the "blank," 2 mL of DI water are added. 2 mL of each standard are added to the corresponding vials. If the wastewater sample is tested at full strength, then 2 mL is added to the corresponding vial. If dilution is required, then serial dilutions are performed and 2 mL of the diluted sample are added to the corresponding vial. Each vial is mixed well and placed into the reactor block for two hours. After two hours, the vials are removed from the block to a cooling rack for about 15 minutes. The colorimeter is set and calibrated per the specific instructions for that unit (i.e., proper wavelength, blank and standards) and each vial is placed in the unit and the COD concentration read. If the sample was diluted, the corresponding multiplication is made.

3.2.2.2 Nitrates analysis

Samples with variant pH were adjusted to a pH of 5 with sulfuric acid. A total number of 36 samples were analyzed per season. GalleryTM Discrete Analyzer was used for the water samples analysis. The instrument work-station had a full graphical user-interface software (Kunene et al., 2018). The software provided dependable control over the analysis process and easy access to advanced functions. The instrument had an application range of 0.0 to 10.0 mg/L . The principle of the instrument was based on the reduction of nitrate to nitrate with hydrazine sulfate (Kunene et al.,2018). Nitrate ions are produced and those originally present are determined by diazotization with sulphanilamide and coupling with N (1 naphthy)-etheynediamine hydrochloride. The coloured azo dye was measured at 540 nm. This reaction measured Total Oxidized Nitrogen-TON. Nitrate was determined by subtracting Nitrite from TON. Daily calibration was performed using 0-5.0 mg/ L standards which the instrument prepared from the 20 mg /L working stock solution.

Sodium hydroxide was prepared by dissolving about 0.8 g NaOH in 100mL of distilled water. The reductant was prepared by dissolving 0.325 g of hydrazine sulphate ($N_2H_4SO_4$) in 400 mL distilled water. From the resultant solution, 0.75 mL of copper sulphate solution (obtained by dissolving 0.78 g $CuSO_4$.5H2O in 200 mL of volumetric flask) and 5ml of zinc sulphates (prepared by dissolving 9.0 g zinc sulphate in 200 mL volumetric flask) was added and diluted with distilled water to the final volume of 500 mL. Colour reagent were prepared by carefully adding 50 mL of concentrated phosphoric acid (H_3PO_4) to 500 mL of distilled water. 5 g of sulphanilmide was completely dissolved before adding 0.25g N-(1-naphthyl)-ethylnediamimine dihydrochloride. The mixture was then diluted to 100 mL

volumetric flask with distilled water. To prevent the decomposition of the solution, was stored in an amber bottle and kept in a refrigerator at 2-8 °C. 25 mL aliquot of the sample was placed into a clean 150 ml sample tube and filtered with 0.45 μm filter paper. The filtered samples were placed in racks and inserted into the instrument. Working instructions as stated by the manufacturer were followed.

3.2.3 Health risk assessment on river water

According to International Agency for Research on Cancer (IARC 2010), human health risks can be classified as carcinogenic and non-carcinogenic risks [158, 170-175]. Carcinogenic risks are defined as health effects that are caused by cancer-causing pollutants, whereas non-carcinogenic risks are those effects that are brought by non-carcinogens[158, 170-175]. Human health risks can be estimated using different indices such as average daily dose (ADD) and hazard quotient (HQ)[170, 176, 177]. Nitrates are classified as non-carcinogens implying that only non-carcinogenic risks is assessed. It has been reported that human being is exposed to nitrates and other pollutants through pathways such as inhalation, ingestion of contaminated water and dermal contact [173-176, 178]. In this study, the human health risk assessment from non-carcinogenic exposure caused by the intake of nitrate in river /stream water through the ingestion pathway was performed for men, women and children. The average nitrate concentrations in Umlaas River and Sterkspruit Stream were used. The exposure in the study area was assessed using average daily dose (ADD) of nitrates ingested from river/stream water [170, 172, 174-176].

$$ADD = \frac{CPW \times IR \times ED \times EF}{ABW \times AET} \quad (1)$$

Where ADD is the average daily dose (mg/kg/day) of nitrates ingested, CPW is the concentration of nitrates (mg L-1) in river water, IR is the ingestion rate per specified time (L/day), ED is the exposure duration (years), EF is the exposure frequency (days/year), ABW is the average body weight (kg) of a person, and AET is the average exposure time (years).

Table 1 Exposure or health risk assessment parameters

Parameters	**Men**	**Women**	**Children**
Ingestion rate (IR, L/day)	2.0	2.0	1.0
Exposure duration (ED, years)	60	67	12
Exposure frequency (EF, days/year)	365	365	365

Average body weight (AWB, kg)	65	60	20
Average exposure time (AET, days)	21900	24455	4380

In addition to ADD, the non-carcinogenic hazard quotient (HQ) model suggested by the United States Environmental Protection Agency was used conduct the health risk assessment [178-181] The HQ value is calculated according to equation 2 [173, 179-181]:

$$HQ = \frac{ADD}{RfD} \quad (2)$$

where RfD is the reference dose for non-carcinogenic risk (1.6 mg/kg/day). According to USEPA, HQ < 1 reveals implausible adverse health impact to the exposed humans, whereas HQ ≥ 1 suggests that there is a possibility that non-carcinogenic impacts might occur [180, 181].

3.2.4 Assessment of water quality

The assessment of water quality was estimated using water quality index (WQI). WQI is known as the single number obtained mathematically from the conversion of large data of water quality [182]. The following steps were used to calculate the WQI:

The relative weight (RWi) is calculated with following equation:

$$RWi = AWi / \sum AWi \quad (1)$$

Where, RWi = Relative weight and AWi = Assigned weight

The quality rating scale for each parameter is conveyed with the following equation:

$$qi = (ci/si) * 100 \quad (2)$$

Where, qi = Quality rating for i^{th} parameter, si = permissible standard for i^{th} parameter set by the WHO and ci = Concentration of i^{th} chemical parameter of water sample (mg L^{-1})

Sub index (SIi) for each parameter is calculated by equation:

$$Sli = RWi * qi \quad (3)$$

Where, SIi = sub index of i^{th} parameter and qi = rating based on concentration of i^{th} parameter

WQI is calculated using the following equation:

$$WQI = \sum Sli \quad (4)$$

According to literature water quality can be divided into five categories based on the value of WQI [152, 182, 183]: Category 1: excellent (< 50); Category 2: good (50 ~ 100); Category 3:

poor (100 ~ 200); Category 4: very poor (200 ~ 300), and Category 5: (> 300) unsuitable for drinking.

3.3.6 Principal Component Analysis (PCA)

The PCA was used to assess the key parameters that could contribute to the variability in the data set (Ochieng et al. 2020). During PCA analysis, principal components (PCs) with eigenvalues more than one were retained because literature states that those explaining the highest total variability in water quality (Ochieng et al., 2020).

3.3 RESULTS AND DISCUSSION

3.3.1 Physico-chemical parameters

Physico-chemical parameters were monitored as they provide an indication regarding the status of quality of water. Results attained such as COD, pH, TSS, and EC are summarized in Table 3.1. For Umlaas River, the mean EC values varied from 30.8-39.0, 24.2-333, 23-45.9 and 41.9-44.5 S m^{-1} in summer, autumn, winter and spring, respectively. In Sterkspruit Stream, the average EC values in summer, autumn, winter and spring were found in the ranges of 52.3-189, 76.0-177, 136-183 and 128-195 mS m^{-1}, respectively. For WWTP outfall, the mean EC values varied from 151-279, 191-221, 245-273 and 242-286 S m^{-1} in summer, autumn, winter and spring, respectively. In general, EC values decreased from the WWTP outfall to the Sterkspruit stream which was followed by further reduction in Umlaas River. This general trend was expected as wastewater is known to contain high amounts of dissolved salts which is related to higher EC. Therefore, both the water from the stream and the river played a role of a diluent in this case. The monitoring of water quality in Umlaas River is essential as this river is accessible to the livestock and local communities. The results attained were generally below the permissible value of 70 mS m^{-1} set by DWAF for domestic use, with the exception of EC values found in autumn. Results for the WWTP outfall and the stream fall within the permissible value of 600 mS/m for domestic use set by the WHO. In the case of river water, EC values found in this study are lower than those reported for other South African rivers located in Durban and Ladysmith with values in the ranges of 331-777 [184] and 28.6-43.8 mS m^{-1} [185].

pH is one of the important physicochemical parameters of water as it tends to have an influence on human health and aquatic life since most of the metabolic activities are pH dependent [170, 178]. The pH values found in all samples were within the pH range of 5 to

9.7 recommended for South African drinking water (SANS, 2015). Based on the results in presented in Table 3.1, there are no noticeable seasonal variabilities.

The total suspended solids in Umlaas River were reported to be 5.00-27.6, 5.25-50.3, 2.67-7.33 and 5.81-11.8 mg L^{-1} during summer, autumn, winter and spring season, respectively. For Sterkspruit Stream, the TSS values during summer, autumn, winter and spring ranged from 28.3-104, 12.5-30.3, 5.33-16.8 and 15.4-22.0 mg L^{-1}, respectively. The TSS for WWTP outfall were found to be 6.00-14.5, 7.25-66.3, 7.67-15.7 and 22.3-56.5 mg L^{-1} in summer, autumn, winter and spring, respectively. It was observed that the lowest TSS were found during the winter and spring season in Umlaas River. In fact, TSS for other samples as well were generally low during the winter season which is regarded as dry season with no or limited rainfalls in South Africa. As such, the variations of TSS in water samples across different seasons could be influenced by the introduction of clay and soil particles into the water bodies due to surface runoffs during rainy periods. This has a potential to increase the amount of TSS present in receiving water bodies.

COD is defined as the total amount of oxygen required to completely oxidize organic matter present in aquatic samples, thereby, resulting in the formation of water and carbon dioxide [186]. COD is one of the parameters used to measure the extent of pollution in the aquatic systems. Based on this description, COD value is related to the amount of organics present in water. Results of the present study (Table 3.1) show some variations across different seasons in the CODs found in the investigated study sites. In Umlaas River, the highest COD concentrations were recorded in autumn, while the highest values in Sterkspruit Stream were found in spring. In addition, the COD concentrations in Sterkspruit Stream and Umlaas River were above permissible limit of 4 mg L^{-1} set by WHO for drinking purposes. In the WWTP outfall, COD values varied as follows; autumn (53.3-80.9 mg L^{-1}) < summer (60.0-81.5 mg L^{-1}) < winter (84.3-107 mg L^{-1}) < spring (104-184 mg L^{-1}). This means the high COD levels found in spring for the WWTP outfall contributed to higher levels observed in Sterkspruit Stream during the same season. Importantly, the COD levels found in Hammarsdale WWTP outfall were generally below the acceptable wastewater discharge limit of 120 mg L^{-1} with one exception observed during the month of November. In a previous South African based study, the physico-chemical characteristics of the WWTP receiving watershed affected the water quality negatively due to the discharge of inadequately treated effluents [164]. Therefore, the amount of organics in the effluent of Hammarsdale WWTP are likely to be the major influence of COD levels in Sterkspruit stream and Umlaas River. However, a highest value of 536 mg L^{-1} was found in Umlaas River. This value and 470 mg

L^{-1} both found in autumn were higher than the levels reported in other studies [187]. In Asa River (Nigeria) and Ramganga River (India), the COD levels did not exceed 58.4 mg L^{-1} [187] and 55.5 mg L^{-1} [137], respectively. This means a routine monitoring of the South African Umlaas River is urgently required with possible remediation plans to reduce the amount organics that could be polluting this river.

Table 3.1:Physico-chemical parameters of water samples

Season	Month	pH			EC (mS m^{-1})			COD (mg L^{-1})			TSS (mg L^{-1})		
		Umlaas River	Sterkspruit Stream	WWTP outfall	Umlaas River	Sterkspruit Stream	WWTP outfall	Umlaas River	Sterkspruit Stream	WWTP outfall	Umlaas River	Sterkspruit Stream	WWTP outfall
Summer	December	7.59	7.45	7.92	38.2	52.0	279	3.00	46.7	72	27.6	104	9.00
	January	7.68	7.65	7.57	39.0	86.3	151	29.0	64.0	60	5.33	43.3	6.00
	February	7.82	7.77	7.87	30.8	186	256	66.5	57.3	81.5	5.0	28.3	14.5
Autumn	March	7.66	7.65	7.73	24.2	76.1	221	<30	78.0	80.8	6.75	30.3	9.25
	April	7.38	7.34	7.88	144	129	191	536	117	64.5	5.25	12.5	7.25
	May	7.77	7.81	7.82	333	177	218	470	58.3	53.3	50.3	32.3	66.3
Winter	June	7.78	7.74	7.96	32.4	136	245	<30	60.7	84.3	7.33	5.33	7.67
	July	7.72	8.03	7.97	40.0	183	265	<30	84.0	101	3.80	16.8	15.3
	August	7.72	7.83	7.97	45.9	155	273	<30	77.7	107	2.67	16.7	15.7
Spring	September	7.69	7.53	7.90	41.9	128	261	56.0	56.7	104	11.7	17.7	22.3
	October	7.95	7.72	7.93	47.0	163	242	98.0	101	105	5.81	15.4	34.0
	November	7.54	7.87	7.77	44.5	195	286	77.3	97.6	183	11.8	22.0	56.5

3.3.2 Nitrate concentrations in water samples

Nitrates were found in all the investigated water samples (Table 3.2). As seen in Table 3.2, the nitrate concentrations ranged from <0.1-9.22, 0.11-21.4 and <0.34-28.1 mg L^{-1} in Umlaas River, Sterkspruit Stream and Hammarsdale WWTP outfall, respectively. This means all the detected nitrate concentrations were below the guideline value of 50 mg L^{-1} set by WHO for safe drinking water. However, it should be noted that the South African guideline value for nitrates in the public water supplies is set at 12 mg L^{-1} (SANS, 2015). In some cases, the nitrates levels exceeded this value. In the case of Hammarsdale WWTP outfall and Sterkspruit Stream, higher nitrate concentrations were observed at the fall of summer (in February) and in autumn (March and April. This means there was no direct correlation between the nitrate levels and physico-chemical parameters monitored in this study. Nitrates levels were sometimes higher in the samples from the WWTP outfall. This means various industries discharging their wastewater for treatment in Hammarsdale WWTP could be indirectly the major sources of nitrates in the Sterkspruit Stream and Umlaas River. However, the nitrate levels in Umlaas River were higher than in other samples during the months of November and January which means there are other sources of nitrates in the river. Overall, the variations in nitrate concentrations observed over the study period might be due to meteorological conditions such as rainfalls and droughts which are related to seasonal changes. According to the literature, seasonal variations in nitrate concentrations are normally associated with the phases of vegetation and animal growth in the aquatic ecosystem. This is because changes in seasonal temperatures regulates the biological processes that determine nitrogen cycling flora and fauna [79]. When compared to nitrates levels reported in other studies[167, 169], it can be concluded that the sites sampled in this work were not heavily polluted. The maximum concentrations of 55 and 50 mg L^{-1} (mean value) were found in Thame River (UK) [169] and Yellow River (India) [167], respectively.

Table 3.2: Concentrations of nitrates in detected in water samples across different seasons.

Season	Month	Concentration (mg L^{-1})								
		Umlaas River			Sterkspruit Stream			WWTP outfall		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Summer	December	0.35	0.66	0.55	0.87	2.80	1.53	0.36	0.36	0.36
	January	< 0.34	6.1	2.26	0.34	0.44	0.40	0.4	0.34	0.37
	February	0.1	2.75	1.04	0.50	21.4	7.81	< 0.50	24.11	13.7
Autumn	March	<0.10	1.75	1.04	<0.5	13.2	5.58	<0.50	28.1	10.8
	April	<0.10	0.93	0.63	0.12	2.5	0.99	0.48	20.1	5.7
	May	0.6	1.05	0.83	0.11	1.06	0.68	0.31	1.05	0.64
Winter	June	0.74	1.55	1.06	0.62	1.12	0.79	0.32	0.38	0.36
	July	0.48	0.73	0.63	0.36	0.82	0.59	<0.34	0.66	0.54
	August	0.41	1	0.66	<0.34	0.77	0.66	0.47	0.59	0.52
Spring	September	0.36	0.55	0.47	0.32	1.42	0.74	0.42	0.75	0.61
	October	0.11	1.64	0.55	0.32	1.78	0.69	0.34	1.83	0.98
	November	0.21	9.22	3.47	0.30	2.09	0.83	0.73	1.84	1.29

3.3.3 Human health risk assessment

3.3.3.1 Estimation of average daily dosage (ADD)

The exposure to nitrate through drinking of contaminated water is one of the contributing factors to human health inequalities in various areas. There are no studies that have investigated the non-carcinogenic effects caused by the presence of nitrate in river water samples from the study area. Although the detected nitrate levels did not exceed the maximum permissible level of 50 mg L^{-1} set by the WHO, some concentrations were above the South African guideline value of 12 mg L^{-1}. This prompted investigation on the assessment of human health risks that could be caused by the exposure to the detected nitrate concentrations. To evaluate the health risk in the river water samples, average nitrate concentrations were used to calculate the non-carcinogenic risk in river water samples. To achieve this, ADD data and HQ of nitrate in river water from the study area via ingestion pathway are presented in Figure 3.1 (Table 3A-1) and 3.2 (Table 3A-2), respectively. Figure 1 shows that the ADD values for river samples ranged from 0.014-0.24 mg/kg/day, 0.013-0.26 mg/kg/day and 0.020-0.39 mg/kg/day for men, women, and children, respectively.

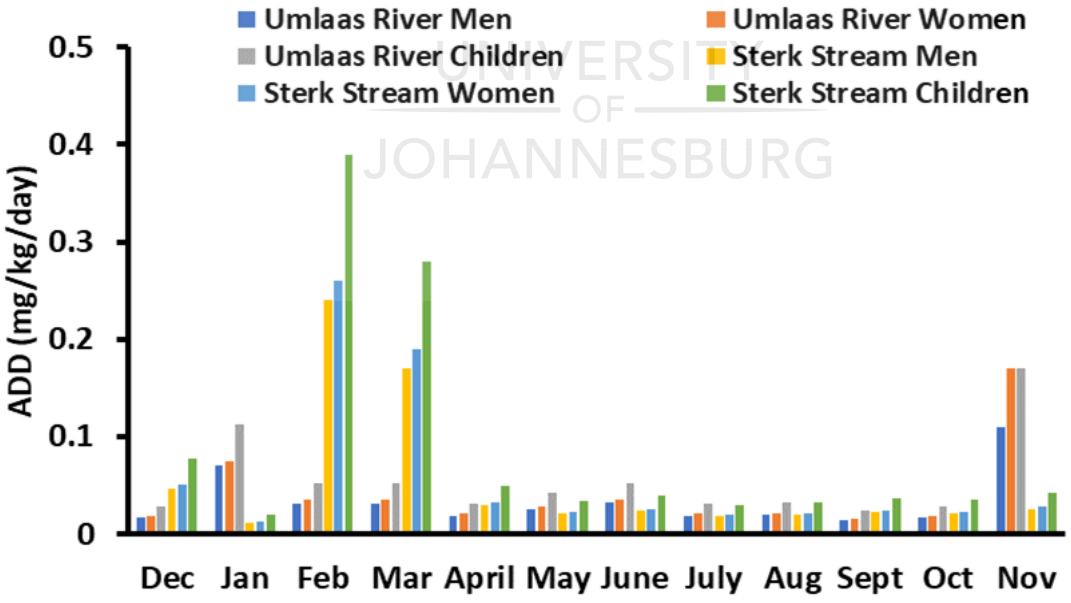

Figure 3.1: Assessment of average daily dosage (ADD, mg/kg/day) values for men, women and children.

3.3.3.2 Assessment of non-carcinogenic risk levels of nitrate in river water

The possible health risks to men, women and children surrounding the study area were from intake of nitrate containing water from the rivers were assessed using hazard quotient (HQ). Figure 2 illustrates HQ values in the river water samples for the injection pathway. The results obtained revealed the values of HQ through oral intake of river water containing nitrate to men, women and children in the study areas ranged from 0.009-0.15, 0.008-0.16 and 0.013-0.24, respectively. According to previous studies harmful effects occur when the HQ value is greater than 1, while HQ values less than one are safe. As seen in Figure 3.2, the HQ values in this study were less than 1 for all children, men and women. These findings suggested that the concentrations of nitrate found in water samples investigated in this study will have no significant health effects on selected age groups. The higher HQ values observed in wet season (between November and March) is an indication that nitrates levels were higher in that period.

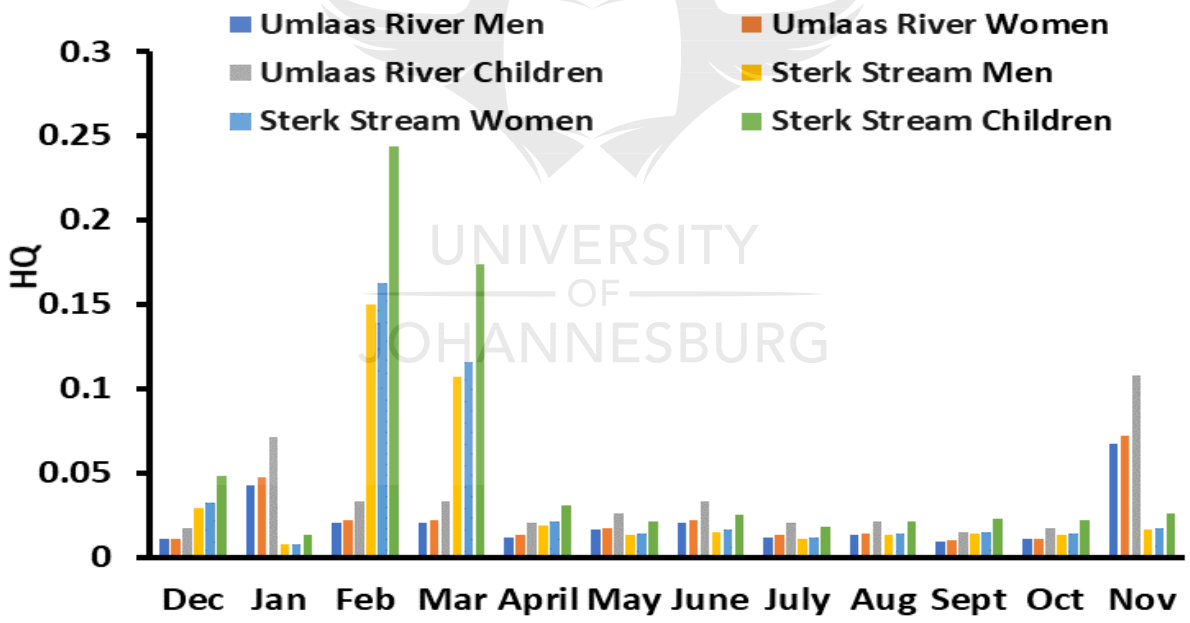

Figure 3.2: Hazard quotient values for nitrate in river water samples from Umlaas River, Sterkspruit Stream

3.3.4 Assessment of water quality

The assessment of river water quality was investigated using WQI based on the WHO drinking water guidelines. The studied water quality parameters assigned weight and relative

weights as well as WHO drinking water guidelines are presented Table 3.3. The weights were assigned according to importance of the each parameter to the water body health [183, 188].

Table 3.3: Relative weight of water quality parameter and WHO drinking water guidelines

Parameters	Drinking water guidelines	Weight	Relative weight
EC (ms/m)	1000	4	0.17
SS (mg L^{-1})	500	5	0.22
COD (mg L^{-1})	10	4	0.17
NO_3^- (mg L^{-1})	50	5	0.22
pH	8.5	5	0.22

The WQI values and water classification are presented in Table 3.4. The calculated WQI values was between 62.5 and 650 for Umlaas River. While for Sterkspruit Stream the values ranged from 140-197. Among these, Table 6 demonstrates the water from Umlass River could be classified as in the category good water for drinking purposes during summer and winter seasons. However, during autumn and spring seasons, the water was classified as poor to unsuitable for drinking water suggesting that during these seasons, the water not good water for drinking purposes. The WQI values for Sterkspruit Stream were above 100 in all seasons and the water was classified as poor water. These results indicates that the effluent from the wastewater treatment plant has an effect on the water quality of the river. The most effective water quality parameters on the determination of WQI for the present study were COD and EC.

Table 3.4: Water Quality Index for river water in the study area

Season	Umlaas River		Sterkspruit Stream (SS)	
	WQI value	Classification	WQI value	Classification
Summer	62.5	Good water	140	Poor water
Autumn	650	Unsuitable for drinking	190	Poor water
Winter	79.4	Good water	177	Poor water
Spring	168	Poor water	197	Poor water

3.3.5 Principal component analysis

A biplot is used to show the correlation between the vectors represented as the redlines and the principal components represented by the x and y axis. Figure 3.3 shows the biplot for

water quality parameters. High and positive correlations between vectors can be seen when vectors are tightly knitted, in this case it was evident that COD and EC have positive correlation with each other and PC1. There was also some correlation between COD, EC and SS. The biplot also showed that the SS and NO_3^- s well as NO_3^- and pH were less likely to be correlated since their vectors formed 90 ° angle at their intersections. The vectors forming large angles at their intersections are said to have negative correlations, in this case the EC-pH, COD-pH, pH-SS, EC-NO_3^-, COD-NO_3^- had large angles meaning these water quality indicators had negative correlations between them. The same trend was observed in all the three sampling sites (Figure 3.3a-c).

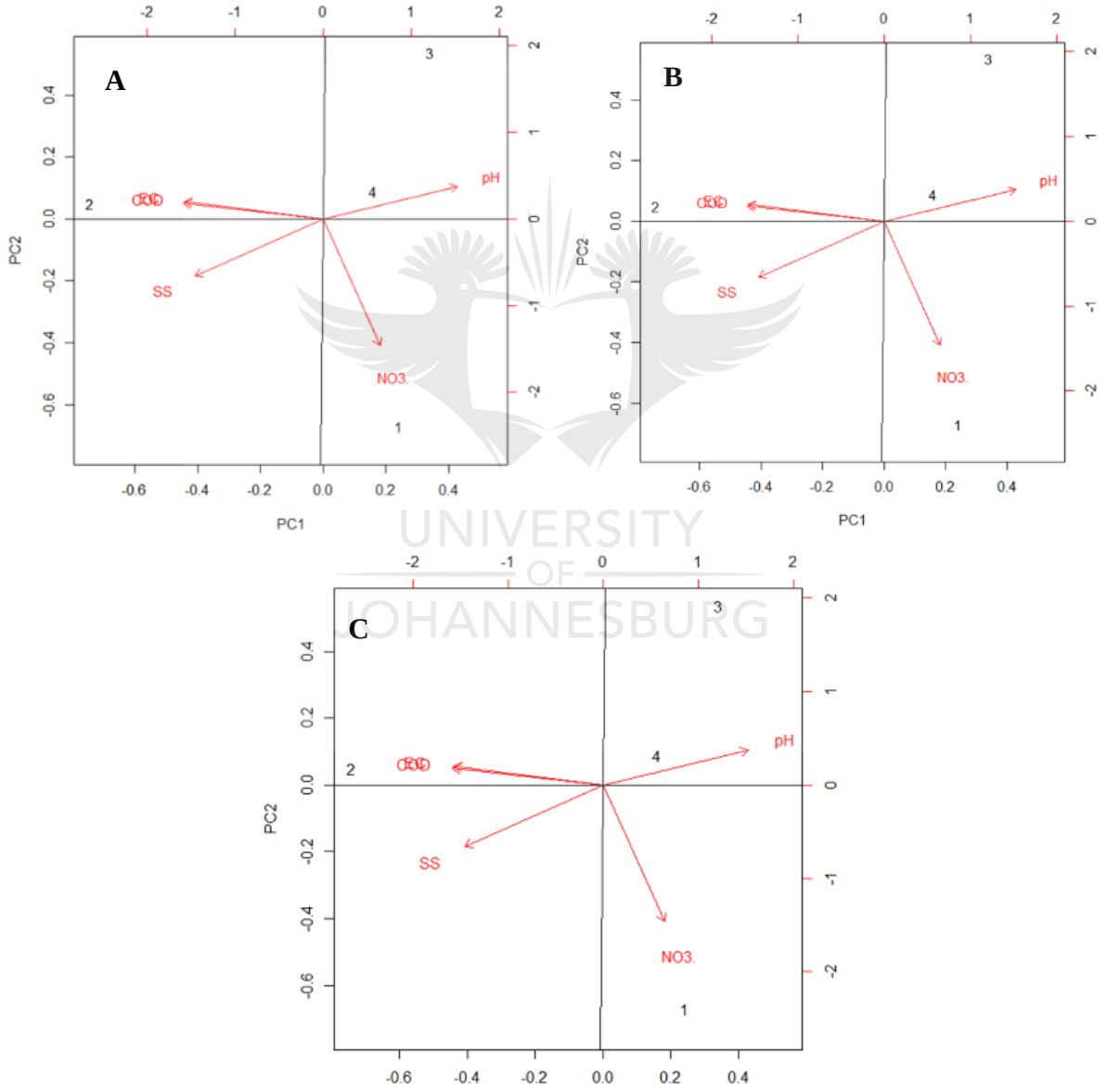

Figure 3.3: PCA correlation biplot of the five physico-chemical variables at a) Hammarsdale Wastewater Treatment Works, b) Umlaas River and c) Sterkspruit Stream. COD =chemical oxygen demand; EC= electrical conductivity; SS = total suspended solids; NO_3^- = nitrates

The vectors for all the seasons intersected at less than 90 ° angles, meaning that there was some correlation between the seasons. In Figure 3.4a & b, spring and winter had higher correlation between them in both the Hammarsdale Wastewater Treatment Works and Umlaas River sites. In contrast, the Sterkspruit Stream (Figure 3.4c), winter, spring and autumn's vectors were tightly knit showing positive correlation between the seasons. Additionally, the three vectors were almost horizontal showing high correlation with PC1.

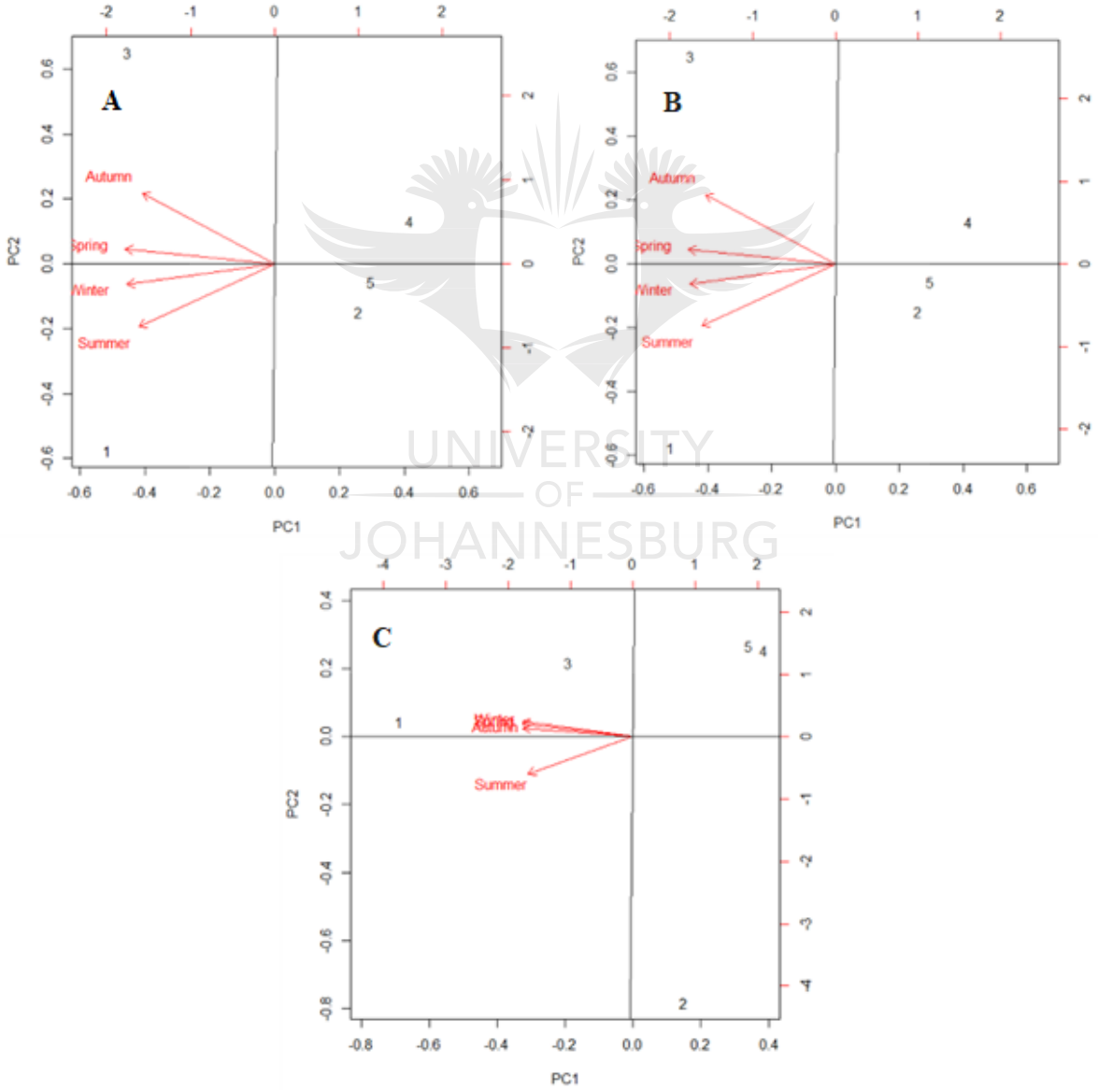

Figure 3.4: PCA correlation biplot for seasonal variability of physicochemical parameters measured water samples collected from a) Hammarsdale Wastewater Treatment Plant (WWTP), b) Umlaas River and c) Sterkspruit Stream

Figure 3.5a shows that the NO_3^- in summer-autumn and winter-spring were correlated, while there was negative correlation between summer-winter, autumn-winter, summer-spring and autumn-spring. Figure 3.5b showed positive correlation between winter, spring and summer while autumn was less likely to be correlated with the other 3 seasons and PC1 for EC. A similar result could be observed for pH in Figure 3.5c. In contrast, summer-winter, summer-autumn, spring-autumn and spring-winter were less likely to be correlated while summer-spring and winter-autumn showed some correlation for SS (Figure 3.5d). The biplot for COD (Figure 3.5e) showed high correlations between summer and winter. The two vectors are also almost horizontal which shows correlation with the PC1. Summer, winter and spring has some correlation, while negative correlations were observed between summer and autumn, winter and autumn, autumn and spring.

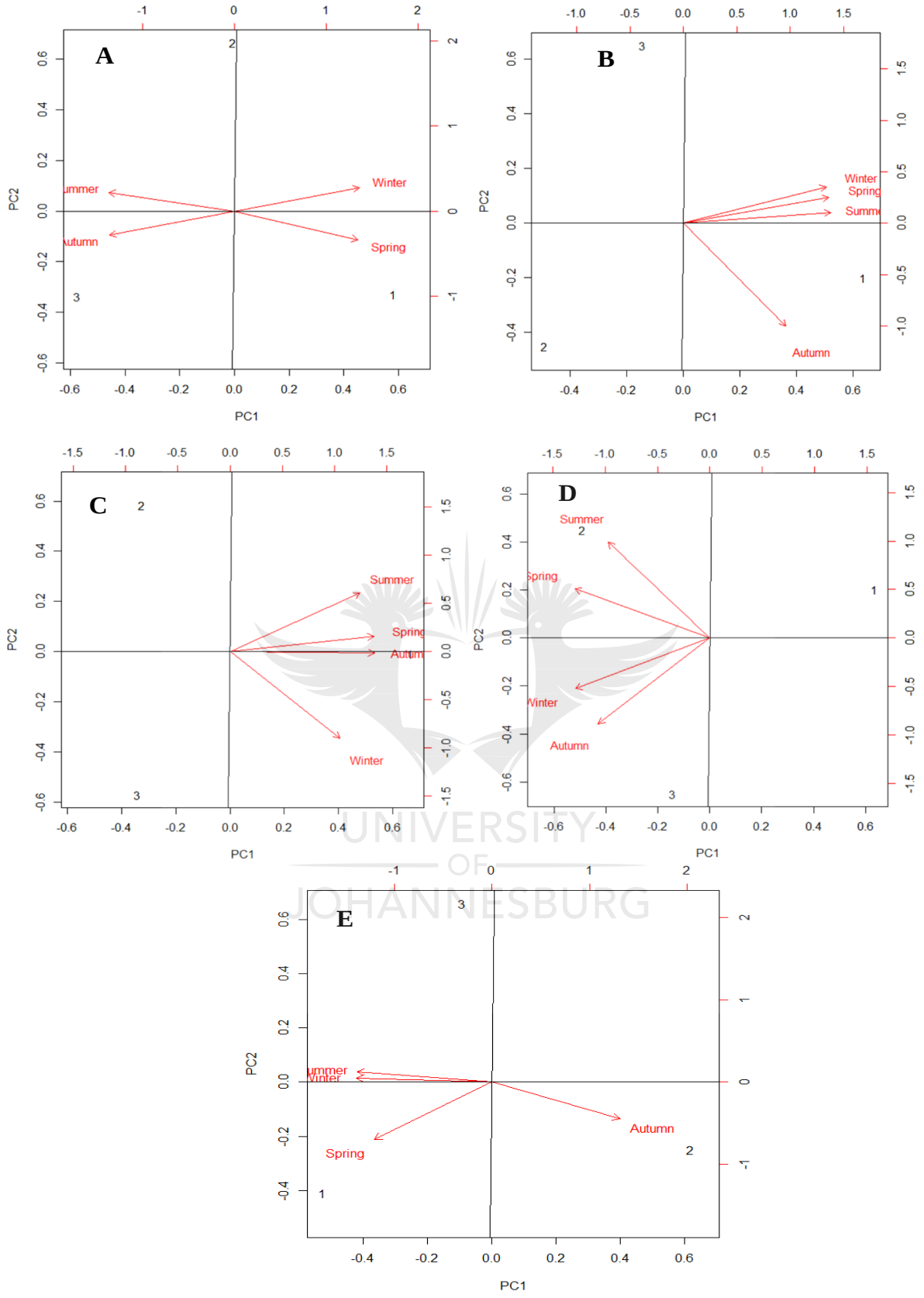

Figure 3.5: PCA correlation biplot of the seasonal variations with respect to a) nitrates, b) electrical conductivity, c) pH, d) total suspended solids and e) chemical oxygen demand

3.4 CONCLUSION

In this study, the status of water quality in a South African public river was monitored through the assessment of phyico-chemical parameters, nitrates, and human health risks. The nitrates levels in all samples did not exceed the maximum guideline value of 50 mg L^{-1} set for the safe drinking water by the WHO. Based on the environmental concentrations found for nitrates, there were no significant health issues that could affect men, women and children with access to the investigated water resources. Generally, the physico-chemical parameters were mostly within the acceptable allowable limits, with few exceptions noted. This was mostly the case for the COD in surface waters indicating a high amount organic matter in these samples. There was no clear correlation between the nitrates levels and physico-chemical parameters. It is therefore evident that the water quality in Umlaas River is not entirely safe for continuous consumption without some form of treatment. However, further research is required to identify the exact causes of high COD and resume the possible remediation processes to improve water quality. Moreover, The most effective water quality parameters on the determination of WQI for the present study were COD and EC and these findings were in agreement with PCA.

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CHAPTER 4: GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

4.1 GENERAL CONCLUSIONS

In this study, physico-chemical properties and nitrate concentrations were monitored in Umlaas River and Sterkspruit Stream over a one year period. The same properties and nitrates levels were monitored in Hammarsdale WWTP in order to understand the role played by this water treatment facility in the contamination of river water. Physico-chemical properties monitored included the pH of water, COD, total suspended solids and electrical conductivity. The maximum permissible levels set by the WHO and the South African regulatory authorities for these parameters in safe drinking water were used as guideline values when assessing the status of the sampled water quality. In general, the physico-chemical parameters were mostly within the acceptable allowable limits, with few exceptions noted for COD values. High COD values which exceeded the value of 4 mg L^{-1} set by the WHO were linked to the amount of organic matter present in water which is likely to be originating from the surrounding industries. Throughout the four seasons of the year, there was no clear correlation between the nitrates levels found in water and the physico-chemical parameters. Due to high COD values in most cases, it was therefore evident that the water quality in Umlaas River was not entirely safe for continuous consumption without some form of treatment. The nitrates levels in all samples did not exceed the maximum guideline value of 50 mg L^{-1} set for the safe drinking water by the WHO. However, some detected nitrate concentrations exceeded a maximum permissible level for nitrates in the public water supplies set at 12 mg L^{-1} in South Africa. This presented a need to compute the human health risk assessments. In this context, there were no significant health risk issues that could affect men, women and children that have access to the investigated water resources.

4.2 RECOMMENDATIONS FOR FUTURE WORK

In most cases, COD values exceeded the maximum permissible level in water set by the WHO. High COD values are associated with high levels of organic matter in water. In addition, the occurrence of nitrates in water was evident in this study. Sometimes, the nitrate levels in water samples exceeded the South African guideline value. Further research is required to alleviate these problems. Future research should involve the following:

- The source of organics in water samples should be identified. Once this is done, it would be easy to safeguard the contamination of river water by the organics, hence the COD levels will be kept at minimal.
- Continuous detection of nitrates is one of the major concerns. One of the major sources of nitrates in environmental waters is the application of nitrogen containing fertilizers on agricultural fields. The water run-off during the rainy days channels nitrates into the nearby water resources. However, nitrates were still present in the study sites during the dry season (winter) and when no agricultural activities were taking place. This means there are other sources of nitrates in these study sites. And these sources should be identified in future, so that proper remediation strategies can be planned and implemented.
- Importantly, the communities residing along the polluted rivers need to perform a clean-up step prior to the consumption of Umlaas River water. This could be done targeting the removal of organics and nitrates in water.
- Finally, more environmental monitoring studies are required to fully understand the extent of water pollution. This could be done over the extended period of time, rather than performing them just over a one year period.

APPENDIX

Table 3A.1: Assessment of average daily dosage (ADD, mg/kg/day) values for men, women and children.

Month	Umlaas River			Sterk Stream		
	Men	Women	Children	Men	Women	Children
Dec	0.017	0.018	0.028	0.047	0.051	0.077
Jan	0.07	0.075	0.113	0.012	0.013	0.02
Feb	0.032	0.035	0.052	0.24	0.26	0.39
Mar	0.032	0.035	0.052	0.17	0.19	0.28
April	0.019	0.021	0.032	0.03	0.033	0.05
May	0.026	0.028	0.042	0.021	0.023	0.034
June	0.033	0.035	0.053	0.024	0.026	0.04
July	0.019	0.021	0.032	0.018	0.02	0.03
Aug	0.02	0.022	0.033	0.02	0.022	0.033
Sept	0.014	0.016	0.024	0.023	0.025	0.037
Oct	0.017	0.018	0.028	0.021	0.023	0.035
Nov	0.11	0.17	0.17	0.026	0.028	0.042

Table 3A.2: Hazard quotient values for nitrate in river water samples from Umlaas River, Sterkspruit Stream

Month	Umlaas River			Sterk Stream		
	Men	Women	Children	Men	Women	Children
Dec	0.011	0.011	0.017	0.029	0.032	0.048
Jan	0.043	0.047	0.071	0.008	0.008	0.013
Feb	0.02	0.022	0.033	0.15	0.163	0.244
Mar	0.02	0.022	0.033	0.107	0.116	0.174
April	0.012	0.013	0.02	0.019	0.021	0.031
May	0.016	0.017	0.026	0.013	0.014	0.021
June	0.02	0.022	0.033	0.015	0.016	0.025
July	0.012	0.013	0.02	0.011	0.012	0.018
Aug	0.013	0.014	0.021	0.013	0.014	0.021
Sept	0.009	0.01	0.015	0.014	0.015	0.023
Oct	0.011	0.011	0.017	0.013	0.014	0.022
Nov	0.067	0.072	0.108	0.016	0.017	0.026